

Allergen-Specific IgE Measured by a Continuous Random-Access Immunoanalyzer: Interassay Comparison and Agreement with Skin Testing

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Background: Our aims were to evaluate the performance of a fully automated system for measuring circulating allergen-specific IgE (sIgE) against an established *in vitro* assay and to assess the system's diagnostic accuracy against objective clinical criteria for identifying sensitization to specific allergens.

Methods: Using both the IMMULITE[®] 2000 Allergy system (IML) and an assay based on the widely used ImmunoCAP[®] technology (CAP), we measured sIgE in serum samples from 169 persons with suspected allergies to airborne or insect venom allergens. Skin testing outcome served as the clinical comparison method.

Results: Interassay classification agreement between the IML and CAP, relative to the usual allergen-specific IgE cutoff of 0.35 kIU/L, ranged from 76% (yellow jacket venom) to 95% (orchard grass); agreement was 88.3% for all 9 allergens combined (766 results). The 90 discordant results, when resolved by skin testing, showed better agreement with the IML (72%) than with the CAP (28%). Compared with skin testing, for each of the 9 allergens studied, the area under the ROC curve was at least as large for the IML as for the CAP, reflecting in part the more extensive working range of the IML (0.10–100 kIU/L vs 0.35–100 kIU/L for CAP).

Conclusion: Laboratory testing for sIgE can be performed on a fully automated, random-access system with an extended working range and with diagnostic accuracy for representative allergens equivalent to or better than that of the semiautomated CAP technology.

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Atopic allergic conditions such as asthma, allergic rhinoconjunctivitis, and atopic eczema, as well as other immediate-type allergies, are characterized by an increase in circulating allergen-specific IgE (sIgE)⁴ antibodies (1). The prevalence of IgE-mediated allergic diseases, which can almost be considered a modern epidemic, has increased dramatically in industrialized countries (2). This increase has created a greater need for early diagnosis to direct early intervention that may prevent disease progression and the development of chronic illness.

The diagnosis of IgE-mediated allergic diseases is routinely based on 4 types of evidence (3). Three are part of the clinical work-up for allergy, which involves a detailed patient history and physical examination, skin testing, and in certain cases, challenge testing with a suspected allergen. The fourth type is a laboratory procedure, most commonly *in vitro* determination of circulating serum IgE antibodies specific for allergens.

Over the last 3 decades, laboratory testing for sIgE has become widely accepted worldwide as part of the diagnostic arsenal (4–6). Originally described in 1967 by Wide et al. (7), the radioallergosorbent test (RAST) became the first routine technique for the determination of sIgE antibodies in serum. Subsequent second-generation methods (8) had improvements such as greater speed, higher binding capacity, and use of nonisotopic labels, as well as reporting of sIgE concentrations in a continuous scale (kIU/L) standardized to the WHO International Reference Preparation for IgE (2nd IRP 75/502).

In the absence of a recognized reference method for *in vitro* sIgE measurement, the Pharmacia second-generation ImmunoCAP[®] technology has become a quasi-standard because of its widespread use, analytical reliability, and the generally adequate correspondence of its results—on

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⁴ Nonstandard abbreviations: sIgE, allergen-specific IgE; DPC, Diagnostic Products Corporation; IML, IMMULITE 2000 Allergy system; CAP, AutoCAP system based on ImmunoCAP technology; CDA, cumulative distribution analysis; CI, confidence interval; and AUC, area under the curve.

a positive/negative basis—with the results of skin testing (4–6, 8). Nonetheless, second-generation test systems for sIgE have limitations with regard to sample handling, turnaround time, laboratory integration, and personnel requirements. In addition, limitations of the solid-phase immobilization of allergens have been addressed (9, 10). Third-generation systems have finally been developed (10, 11); these systems are based on demonstrated technology, have considerably shorter turnaround times, and use chemiluminescence. The first such assay to become available is the Diagnostic Products Corporation (DPC) IMMULITE® 2000 Allergy (IML) (11, 12), which implements routine sIgE testing on a family of systems already well established in clinical chemistry for performing immunoassays and immunometric assays (13–15).

Here we examine the performance of the third-generation IML assay by comparing IML results with results obtained with a second-generation assay based on the ImmunoCAP technology (as a laboratory comparison method) and comparing the results obtained by these 2 *in vitro* assays in parallel with results obtained by skin testing (as a clinical comparison method). The study protocol was applied to a spectrum of aeroallergens and insect venom allergens representative of the core high-volume workload in our laboratory.

Materials and Methods

PARTICIPANTS

Individuals suspected of having IgE-mediated allergy to aeroallergens and/or insect venoms were recruited prospectively from July 2001 to December 2001 from all those presenting to the Allergy Unit of the Department of Dermatology and Allergy of the Technical University of Munich. Primary suspicion of allergy was based on the patient's clinical history.

The patients constituted 2 representative case streams, depending on the type of allergy suspected. Neither the enrollment of patients nor the selection of data for analysis was influenced by skin testing outcomes or by results obtained with either of the sIgE assays. A total of 230 patients meeting the inclusion criteria agreed to participate in the study. Written informed consent for drawing an additional serum sample for use in the sIgE method-comparison study was obtained from each patient. The inclusion criteria were as follows: age between 10 and 75 years; not taking any medication that could interfere with the results of skin testing; and no autoimmune disease, cancer, or other immunologic disorder (Table 1). In addition to *in vitro* results for circulating sIgE, skin test results were obtained whenever possible. No skin test results could be obtained in cases of dermatographism or of inflamed skin on the forearms, e.g., in patients with acute flare-ups of atopic eczema.

Each patient's final diagnosis was based on skin testing in conjunction with patient history, physical examination, sIgE, and when necessary, a challenge test with the relevant allergen. The diagnoses and their frequencies are

Table 1. Study population.

Enrollment	
Participants enrolled, n	230
Men, n (%)	97 (42)
Women, n (%)	133 (58)
Locale	Munich, Germany
Ethnicity	Caucasian
Final data set	
Allergens studied, n	9
Complete records, ^a n	766
Individuals represented, ^b n	169
Results per individual, n	4.6
sIgE discrepancies, ^c n	90

^a Records containing all 3 values (IML, CAP, and skin testing results) for a given sample and allergen.

^b Number of persons represented in the final database, limited to complete records for the 9 allergens listed in Table 2 of the online Data Supplement.

^c sIgE results <0.35 kIU/L by one assay but not the other. These represent discrepancies relative to the standard positive/negative cutoff for sIgE.

listed in Table 1 of the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol51/issue7/>; for many patients more than one diagnosis applied.

SERUM SAMPLES AND ALLERGENS TESTED

An ~6-mL blood sample was drawn from every participant and labeled appropriately. Each sample was divided into several aliquots and stored at –20 °C until analyzed. Depending on the type of allergy suspected, samples were tested by the 2 *in vitro* sIgE assays either for 7 aeroallergens (3 indoor and 4 outdoor aeroallergens) or 2 insect venoms (Table 2 of the online Data Supplement). The spectrum of the chosen allergens was representative of the core high-volume workload in our laboratory as well as of the geographic region. For all allergens, commercial skin test solutions with demonstrated quality were available.

SKIN TESTING

Skin testing was performed on the volar surface of the patients' forearms by an experienced physician according to recommended guidelines (16, 17). In cases of suspected allergy to indoor and outdoor aeroallergens, skin-prick testing was performed by standard procedures; prick lancets and allergen extracts were obtained from Allergopharma (17). As positive and negative control solutions, respectively, histamine hydrochloride (5 g/L) and physiologic saline were used in all instances. Intradermal testing of patients with suspected insect venom allergies was performed by use of serial 10-fold dilutions of venom extracts (Venomil®; Bencard) with concentrations ranging from 0.0001 to 0.1 mg/L (18). The testing was performed with an insulin syringe and an injection volume of 0.05 mL. Histamine hydrochloride and physiologic saline were used as positive and negative control solutions, respectively. Skin-prick tests were rated positive when the wheal size was ≥3 mm and intradermal tests when the

wheel size was ≥ 5 mm in diameter with a surrounding erythema.

BLOOD TESTING

Circulating sIgE was measured by 2 different methods: the IML from DPC and the AutoCAP[®] FEIA (CAP), a fluoroenzymometric immunoassay from Pharmacia. Allergens for the IML and CAP were obtained from the manufacturers, and all testing was performed according to the instructions of each manufacturer. The main sources for allergen extracts, according to manufacturer information, were Greer and Vespa Laboratories for DPC and Allergen for Pharmacia.

The IML has a working range of 0.10–100 kIU/L. Studies of the analytical performance of the IML assay have been published (11, 12). The assay is designed for the IMMULITE 2000, which is a fully automated, random-access immunoanalyzer. It uses an enzyme-enhanced chemiluminescent detection system and has been validated for a broad spectrum of assays (13–15). It was recently modified for routine sIgE testing (11, 12). The assay combines DPC liquid-allergen chemistry (4, 9) with the IMMULITE bead and wash technology. The ligand biotin is covalently linked to a soluble polymer matrix, which in turn attaches to NH_2^- , OH^- , COOH^- , or SH^- groups on the allergen (9). Through the presence of the identifiable ligand biotin on the soluble backbone but not on the allergen molecule, the IgE binding sites on allergens are preserved. Allergens biotinylated through this indirect procedure and sIgE antibodies bind in liquid phase, thus forming allergen–antibody complexes. These complexes are subsequently captured by the streptavidin-coated beads. A secondary enzyme-linked antibody against human IgE recognizes bound patient IgE. Finally, a chemiluminescent reaction is initiated by substrate addition.

The established CAP system has, in the configuration used here, a working range of 0.35–100 kIU/L. Studies of its clinical and analytical performance have been published (4–6, 8). In contrast to the IML, the CAP assay as used in the present study is a semiautomated procedure, requiring manual distribution of the allergens. The CAP uses the solid-phase ImmunoCAP technology combined with a secondary enzyme-linked antibody that recognizes allergen-bound patient IgE and a fluoroenzymometric detection system. By extrapolation, the CAP system has also been used below the usual 0.35 kIU/L cutoff [reviewed in Ref. (6)].

Results for both systems are expressible in quantitative units, kIU/L IgE, as well as in terms of the traditional spectrum of 7 semiquantitative classes, ranging from class 0 (all results < 0.35 kIU/L) up to class 6 (all results ≥ 100 kIU/L). The IML and CAP are both standardized to the WHO 75/502, as are all second- and third-generation sIgE assays. Moreover, IML and CAP results essentially agreed for the mapping of class boundaries to allergen-specific IgE concentrations, as shown in Table 3 of the online Data

Supplement (8). Total IgE was measured for all patients on both systems according to the manufacturers' instructions.

DATA ANALYSIS

Skin testing was adopted as the objective comparison method for judging the diagnostic accuracy of the 2 *in vitro* sIgE assays. Only complete records with IML, CAP, and skin-testing results were used for the analysis. ROC plots were constructed from smoothed, allergen-specific cumulative distribution analysis (CDA) representations of the skin test-negative and -positive distributions as a function of concentration (19).

Three regression techniques were applied: Deming, OLS Bisector, and a Passing–Bablok procedure (20). Confidence intervals (95% CIs) for proportions and their differences were calculated by Wilson's method (21) and by the method of Newcombe and Altman for paired data (22), respectively. For regression parameters, 95% CIs were calculated by a calibrated percentile bootstrap method (23). The Harrell–Davis technique was used to estimate medians and for smoothing empirical centile curves (24). Data management, statistical analyses, and graphics were implemented in S-Plus 6.1 for Windows (25).

Results

INTERASSAY COMPARISON OF sIGE VALUES

The IML yielded median concentration results for sIgE that were at least as high as those for the CAP for each of the 9 allergens tested (Fig. 1). The IML medians were distinctly higher for cat dander (E1), orchard grass (G3), and yellow jacket venom (I3). Agreement between the IML and CAP results relative to the usual 0.35 kIU/L cutoff for each allergen ranged from 76% for I3 to 95% for G3 and was 88% overall (766 total results combined; Table 4 of the online Data Supplement).

Regression analysis was restricted to mutually explicit results from 0.35 to 100 kIU/L for both assays, 271 of 766 paired results (35%). Values in kIU/L were logarithmically transformed before analysis and then subjected to regression by 3 symmetric regression techniques, each based on a different principle. Several variants of Bland–Altman analysis failed to yield any additional insight (data not shown). As shown in Fig. 2, although results for the $n = 271$ data set are, on average, distinctly higher in the IML than in the CAP, much closer quantitative agreement can be expected near the 0.35 kIU/L cutoff, the concentration of greatest clinical significance. The mean values predicted for the IML at the cutoff for CAP (0.35 kIU/L) by different methods of regression analysis were as follows: 0.344 kIU/L (95% CI, 0.22–0.48 kIU/L) by Deming; 0.44 (0.34–0.55) kIU/L by OLS Bisector; and 0.48 (0.30–0.67) kIU/L by Passing–Bablok/Harrell–Davis regression analysis. At the 0.35 kIU/L cutoff for the IML, the predicted mean values for the CAP were 0.355 (0.27–0.48) kIU/L by Deming, 0.29 (0.23–0.36) kIU/L by OLS

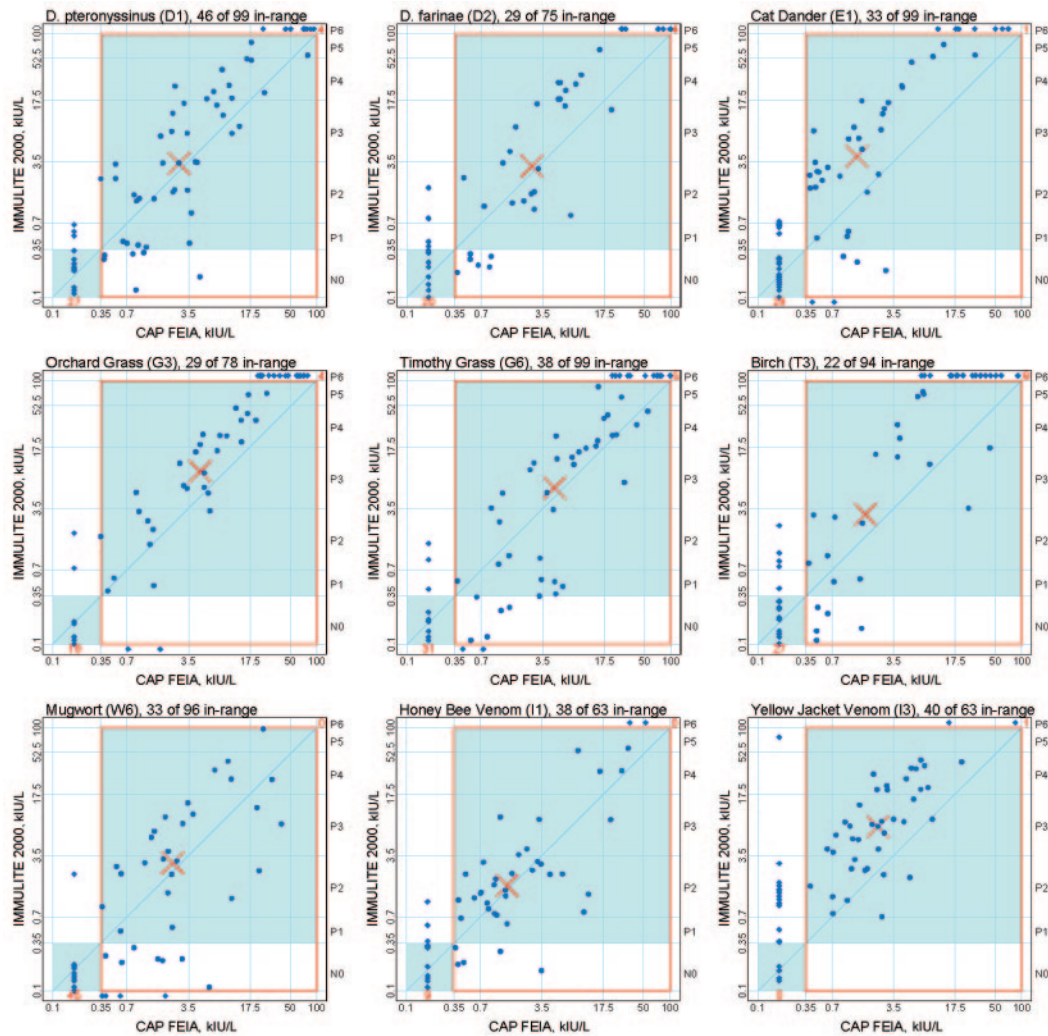


Fig. 1. Scatterplots of IML vs CAP results for each of the 9 allergens, a total of 766 paired sIgE results (169 patients).

The 2 assays share a common mapping from sIgE concentration (kIU/L) to class (0–6), represented by the axis labels and grid lines. The working ranges, i.e., the concentration limits within which the assays can return explicit numeric results in kIU/L (0.35–100 kIU/L for CAP and 0.10–100 kIU/L for IML) are demarcated by the *large rectangle* outlined in *red* within the plotting area. Results out of range by one assay or the other are plotted in the middle of the area (or “gutter”) surrounding this rectangle. The number of mutually explicit results and the total number of results are documented *above* each graph. Shown at the *lower left* and *upper right* of each graph are the number of results simultaneously out-of-range low or out-of-range high by both assays. The *blue diagonal line* represents the line of identity. A *large red x* marks the center of gravity of the mutually explicit sIgE results; i.e., the medians calculated by the Harrell–Davis method.

Bisector, and 0.27 (0.20–0.40) kIU/L by Passing–Bablok/Harrell–Davis regression analysis.

Total IgE concentrations [median, 125 IU/mL (interquartile range, 40–350 IU/mL), as measured by the IML] had no influence on the results of sIgE testing in both the IML and CAP assays (data not shown).

COMPARISON OF sIGE RESULTS WITH SKIN TESTING

The sensitivity, specificity, and agreement relative to skin testing for results obtained by the 2 sIgE assays for each of the 9 allergens studied are shown in Table 2. For the in vitro assays, the usual 0.35 kIU/L cutoff was used (also see Table 5 of the online Data Supplement). Differences between the IML and CAP in terms of their agreement with skin testing were not statistically significant for the individual aeroallergens; however, for the aeroallergen

group as a whole, the difference, favoring the IML, was significant. For both venom allergens, we found a significant difference in favor of the IML, although this was more clear-cut for yellow jacket venom (I3) than for honey bee venom (I1). Overall, for all 9 allergens combined, agreement with skin testing was 86% for IML and 80% for CAP, the difference being significant. The data in the left panel of Fig. 3 show how the 2 sIgE assays compared with respect to agreement with skin testing relative to the 0.35 kIU/L cutoff. The points represent the agreement scores listed for each allergen in Table 2, and the vertical and horizontal lines represent the 95% confidence limits. The points for yellow jacket venom (I3) and mugwort (W6) are labeled; they lie far from the regression line defined by the other 7 allergens. Because the points all lie on or substantially above the line of identity (diagonal

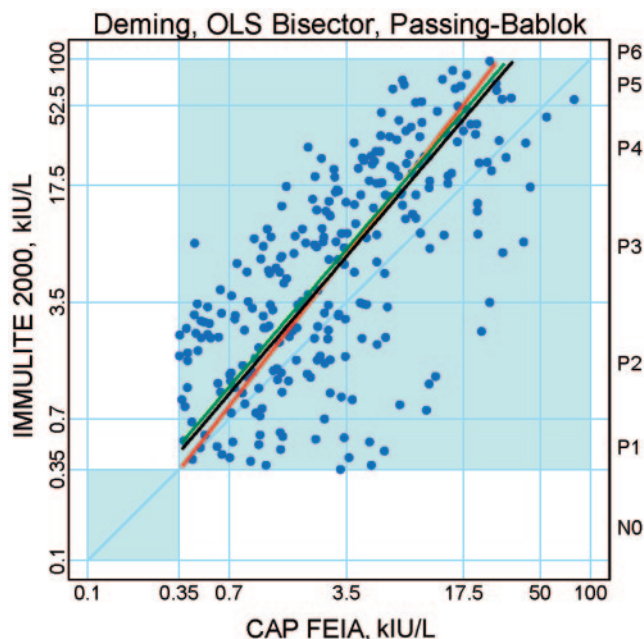


Fig. 2. Regression analysis of the new IML and the established CAP assays, using sIgE concentrations from 271 mutually explicit data points restricted to the 0.35–100 kIU/L concentration range for all 9 allergens combined.

The distribution of individual data points for each of the 9 allergens within the data set used for regression analysis is shown as Fig. 2 of the online Data Supplement. Median concentrations: 5.9 kIU/L in the IML and 2.8 kIU/L in the CAP ($r^2 = 0.462$). *Red regression line*, results for logarithmic regression analysis using orthogonal regression (also known as unweighted Deming regression): IML = $1.29(\text{CAP}) + [0.12 \log_{10}(\text{kIU/L})]$; $S_{y/x} = 0.53$ kIU/L (95% CI, 0.47–0.59 kIU/L); slope, 1.29 (1.16–1.45); intercept, 0.12 (0.01–0.22) kIU/L; CAP = $0.78(\text{IML}) - [0.10 \log_{10}(\text{kIU/L})]$; $S_{y/x} = 0.41$ (0.37–0.45) kIU/L; slope, 0.78 (0.68–0.86); intercept, -0.10 (-0.18 to -0.01) kIU/L. *Green regression line*, results for logarithmic regression analysis using Passing–Bablok 3.1/Harrell–Davis regression: IML = $1.18(\text{CAP}) + [0.22 \log_{10}(\text{kIU/L})]$; $S_{y/x} = 0.50$ (0.45–0.55) kIU/L; slope, 1.18 (1.09–1.29); intercept, 0.22 (0.05–0.33) kIU/L; CAP = $0.85(\text{IML}) - [0.19 \log_{10}(\text{kIU/L})]$; $S_{y/x} = 0.43$ (0.38–0.47) kIU/L; slope, 0.85 (0.77–0.92); intercept, -0.19 (-0.30 to -0.04) kIU/L. *Black regression line*, results for logarithmic regression analysis using OLS Bisector regression: IML = $1.18(\text{CAP}) + [0.18 \log_{10}(\text{kIU/L})]$; $S_{y/x} = 0.50$ (0.45–0.55) kIU/L; slope, 1.18 (1.10–1.27); intercept, 0.18 (0.11–0.25) kIU/L; CAP = $0.85(\text{IML}) - [0.15 \log_{10}(\text{kIU/L})]$; $S_{y/x} = 0.43$ (0.38–0.47) kIU/L; slope, 0.85 (0.79–0.91); intercept, -0.15 (-0.22 to -0.09) kIU/L.

line), it is evident that for the spectrum of allergens studied, the IML agrees overall with skin testing at least as well as does the CAP assay. Discordant results between the 2 sIgE systems were obtained in a total of 90 of 766 instances (11.7%; Table 5 of the online Data Supplement). The frequency with which the skin testing outcome confirmed the sIgE result for one system or the other in these discordant cases, allergen by allergen, are shown in the right panel of Fig. 3. Skin testing results for 72% of the discrepant cases agreed with the IML compared with 28% for the CAP.

ROC ANALYSIS

ROC analyses based on smoothed CDA plots for 3 of the allergens studied, including the 2 allergens highlighted in Fig. 3 (left panel), are shown in Fig. 4 (plots for all 9 allergens are available as Fig. 1 in the online Data Sup-

Table 2. Sensitivity, specificity, and agreement of IML and CAP vs skin testing.^a

Allergen	n	Sensitivity, ^b %	Specificity, ^b %	Agreement, ^{b,c} %
IML				
D1	99	93.8 (83–98)	80.4 (68–89)	86.9 (79–92)
D2	75	84.6 (70–93)	80.6 (65–90)	82.7 (73–90)
E1	99	88.4 (76–95)	87.5 (76–94)	87.9 (80–93)
G3	78	87.0 (76–94)	83.3 (64–93)	85.9 (77–92)
G6	99	82.8 (71–90)	85.4 (72–93)	83.8 (75–90)
T3	94	93.6 (83–98)	87.2 (75–94)	90.4 (83–95)
W6	96	55.8 (41–70)	94.3 (85–98)	77.1 (68–84)
I1	63	91.2 (77–97)	75.9 (58–88)	84.1 (73–91)
I3	63	100 (93–100)	55.6 (27–81)	93.7 (85–98)
CAP				
D1	99	93.8 (83–98)	74.5 (61–85)	83.8 (75–90)
D2	75	79.5 (65–89)	72.2 (56–84)	76.0 (65–84)
E1	99	81.4 (67–90)	83.9 (72–91)	82.8 (74–89)
G3	78	83.3 (71–91)	75.0 (55–88)	80.8 (71–88)
G6	99	82.8 (71–90)	75.6 (61–86)	79.8 (71–87)
T3	94	91.5 (80–97)	85.1 (72–93)	88.3 (80–93)
W6	96	67.4 (53–80)	84.9 (73–92)	77.1 (68–84)
I1	63	85.3 (70–94)	62.1 (44–77)	74.6 (63–84)
I3	63	75.9 (63–85)	77.8 (45–94)	76.2 (64–85)

^a Based on the standard positive/negative cutoff, 0.35 kIU/L, for the sIgE assays.

^b 95% CIs are shown in parentheses.

^c For a graphic comparison of the IML and CAP agreement (raw agreement or efficiency) scores, relative to skin testing, see Fig. 3.

plement). Each CDA plot displays, for a given assay and allergen, the distribution of results (in kIU/L) obtained by the assay for the skin test-positive and -negative samples. Thus, sensitivity and specificity are each plotted against concentration, which served as the basis for constructing a smooth plot of sensitivity against specificity for the assay in the adjoining ROC space representation. For each of the 3 allergens in Fig. 4, and for each of the other 6 allergens (see Fig. 1 of the online Data Supplement), the area under the curve (AUC) for IML was greater than the AUC for CAP (Table 3). Moreover, both the highest clinical sensitivity achievable and the SYM (Q^*) index, the highest sensitivity and specificity jointly attainable, were always higher for IML than for CAP (Table 3).

Discussion

The main goal of the present study was to compare the diagnostic accuracy of sIgE values obtained by IML to objective, well-established in vivo and laboratory methods for identifying sensitization to a representative spectrum of specific aeroallergens and insect venom allergens for patients typical of those encountered by our laboratory on a daily basis. Skin testing for all 9 allergens was adopted as the clinical comparison method to detect allergic sensitization, and the widely used CAP technology as the laboratory comparison method (26).

The present study demonstrates good quantitative agreement of the IML method with the semiautomated

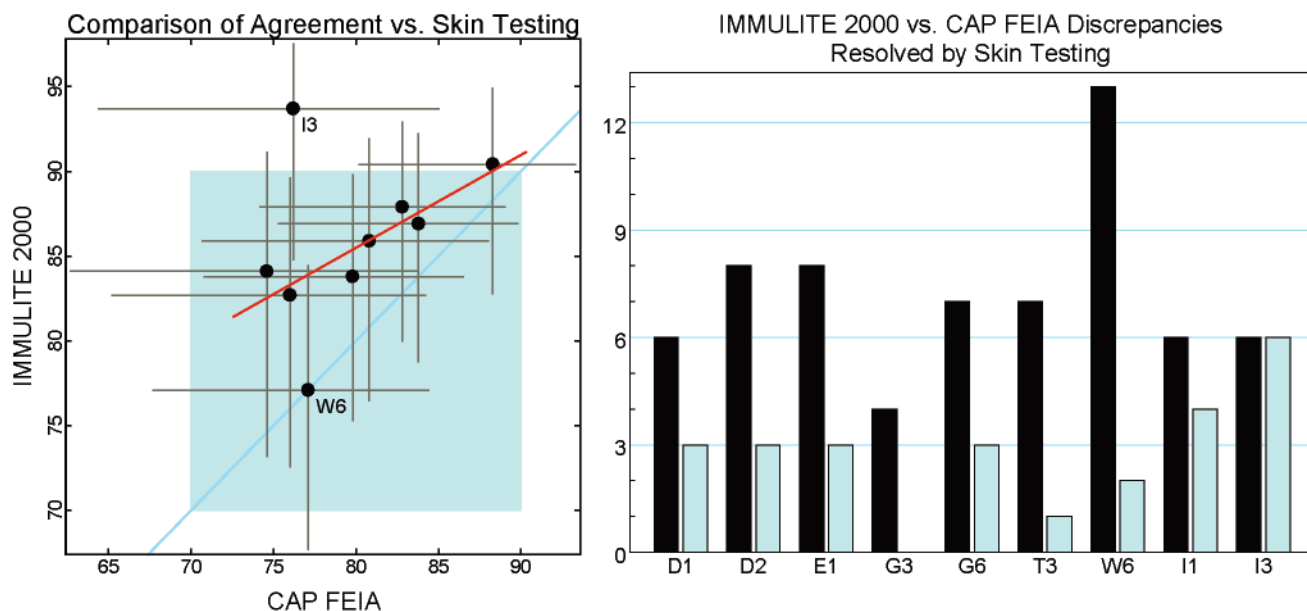


Fig. 3. Comparison of agreement vs clinical skin testing for IML and CAP based on the 0.35 kIU/L cutoff.

(Left), raw agreement or “efficiency” (in percentage; see Table 2) vs skin testing for each of the 9 allergens studied. Results for 2 of the allergens (I3 and W6) are identified as such. The regression line (red) highlights the relationship suggested by the paired estimates of agreement for the remaining 7 allergens. The 95% CIs (see Table 2) are represented by horizontal and vertical lines. The blue rectangle and blue diagonal line indicate the line of identity. (Right), distribution of the 90 sIgE results <0.35 kIU/L by one assay but not by the other, or vice versa. The columns (black, IML; blue, CAP) indicate, for a given allergen and sIgE assay, how many (n) of these discrepant results were in accord with the corresponding skin test result.

CAP technology, which has been considered as a kind of “standard method” for sIgE determination in allergy-testing laboratories. Comparisons in binary terms, relative to the typical 0.35 kIU/L cutoff, also showed a high concordance. The agreement of the results obtained by the IML with the results obtained by skin testing was at least as good as that observed for CAP. Accordingly, our study underscores the importance of comparison with objective clinical methods such as skin testing (6), as it was used here, or even more comprehensive clinical criteria (8) in evaluating the performance of an assay for sIgE, as opposed to relying solely on direct comparison with another laboratory test. The choice of skin testing as the clinical comparison method was based on the following factors: (a) it is an objective and quantifiable clinical method that is considered the most sensitive means of detecting allergic sensitization in a clinical setting (6); (b) it has been considered a clinical standard against which other methods for allergen-specific IgE detection should be compared (6); and (c) the use of skin testing as a clinical comparison method avoids the circularity that would be created by the use of clinical outcome data, which routinely involve data based on sIgE.

Comparisons of the IML and CAP sIgE assays with skin testing were performed both relative to the usual 0.35 kIU/L cutoff and, via ROC analysis, across the entire spectrum of cutoffs achievable for each assay (26). With regard to the 0.35 kIU/L cutoff, IML agrees overall with skin testing at least as well as does CAP and, accordingly, must be judged as acceptable by prevailing standards for

sIgE assays (6). ROC plots depict the relationship between sensitivity and specificity for an assay across all achievable cutoffs, and the statistical analysis of ROC curves is a valuable component of the comparative performance evaluation of 2 or more sIgE assays relative to an independent clinical standard (8, 27–29). The CDA plot, introduced as an alternative to the ROC plot (19), provides both a complementary view of sensitivity and specificity data for quantitative assays and a basis for constructing a smooth, realistic ROC curve respecting the (extended) working range of an assay. The working range of an sIgE assay can be readily identified in a CDA plot, in which one axis represents concentration, but not in an ROC representation, where the 2 CDA trajectories are reduced to a single contour line at the cost of eliminating the concentration axis. The CDA-plot-based ROC analysis for all tested allergens revealed that CAP ROC curves nested cleanly within the IML ROC curves, indicating that the discriminative capacity of the IML, across all achievable cutoffs, is at least as great as that of the CAP. There was less apparent difference between the areas enclosed by IML and CAP ROC curves when we limited our analyses to the region between 0.35 and 100 kIU/L, the working range of the CAP assay (not shown).

Because the quantitative performance of sIgE assays is important (8, 27), we attempted to address issues of method comparisons in quantitative terms (Fig. 1). A substantial fraction of the sIgE concentrations, as measured by the IML, fall in the 0.10–0.35 kIU/L interval. These positive sIgE samples recognized specific allergens

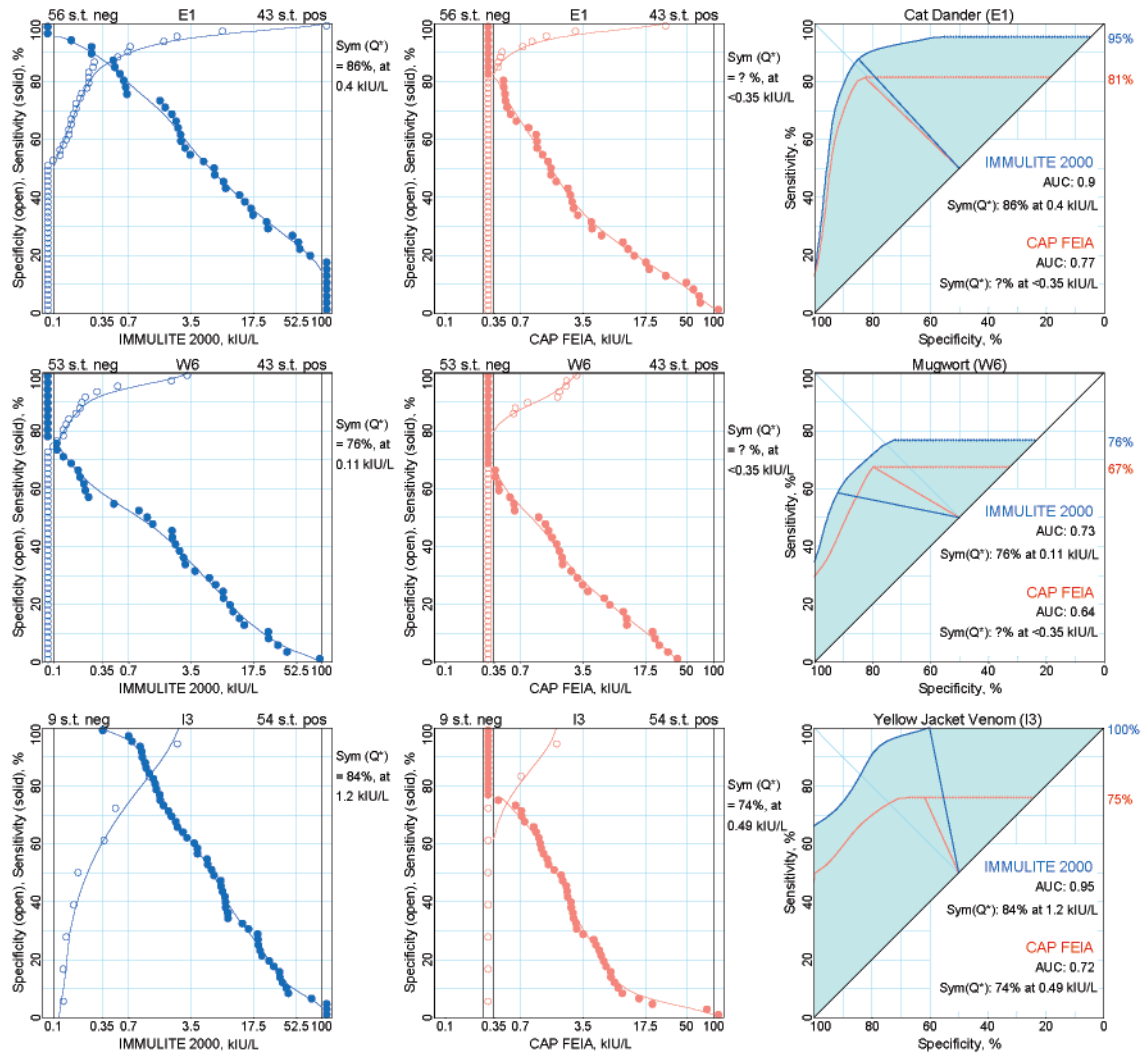


Fig. 4. CDA and ROC plots showing the ability of the IML and CAP assays to indicate the clinical skin test result for 3 representative allergens: cat dander (E1), an indoor perennial inhalant allergen (top row); mugwort (W6), an outdoor seasonal inhalant allergen (middle row); and yellow jacket venom (I3), an insect venom allergen (bottom row).

In each of the 3 ROC plots, the blue line represents the ROC curve for IML, and the red line represents the ROC curve for CAP. The dashed segment of each ROC curve indicates the highest clinical sensitivity achievable for that allergen in this study, given the inherent limitations on the working range of each assay at the low end. As clinical comparison method, a skin prick test (inhalant allergens) or intradermal skin test (venom allergens) was rated positive at a wheal size ≥ 3 mm (skin prick test) or ≥ 5 mm with surrounding erythema (intradermal skin test). In the CDA plot for each allergen and assay, the open circles and filled circles represent the observed skin test-negative (s.t. neg.) and skin test-positive (s.t. pos.) samples, respectively, as a function of the sIgE concentration. The curved lines represent a smoothing of these 2 distributions, via a spectrum of Harrell-Davis centile estimates. In the CDA plots, any results outside the working range of each assay are plotted in a vertical gutter on the left or right. The corresponding ROC plot was constructed from the smooth specificity and sensitivity curves in the CDA plots. The maximum achievable clinical sensitivity of the assay for a given allergen is indicated on the right of each ROC graph. AUCs and SYM (Q*), the highest value that sensitivity and specificity can jointly attain, are also shown for each assay. ?% for the SYM (Q*) indicates that the value could not be determined because of limitations of the lower end of the assay's working range.

in immunoblots and were not dependent on the concentration of total IgE (Ollert et al., manuscript in preparation), which is in accord with the recent findings of Li et al. (11), who also demonstrated that nonspecific IgE at high concentrations (>1000 IU/mL) does not generate false-positive results in the IML. Regression analysis, although frequently used to compare sIgE assays (8), cannot be trusted to improve on this characterization of the relationship between the 2 assays. Our regression analysis, restricted to points in the 0.35–100 kIU/L concentration range covered by both assays (Fig. 2), indicates

that near the usual 0.35 kIU/L cutoff, the quantitative results obtained by the 2 assays are likely to be much closer than the disparity of mean values would suggest.

Complete concordance between in vitro sIgE testing and skin testing cannot be expected: an in vitro assay measures circulating sIgE, whereas skin testing measures cutaneous mast cell reactivity based on assumed cell-bound sIgE. Thus, skin testing, although accepted as the most widely used and most reliable method to detect allergic sensitization in vivo, can aid only in identification of the disease status of the patient and is not a substitute

Table 3. ROC curve statistics for IML and CAP vs skin testing.

Allergen ^a	n	AUC			Maximum sensitivity, ^b %			SYM ^c		
		IML	CAP	Difference	IML	CAP	Difference	IML	CAP	Difference
D1	99	0.917	0.851	0.066	97.9	93.5	4.4	85.6	79.6	6.0
D2	75	0.897	0.773	0.124	94.9	79.5	15.4	82.4	78.7	3.7
E1	99	0.897	0.774	0.123	95.3	81.4	13.9	86.2	NA ^d	NA
G3	78	0.889	0.807	0.082	92.6	83.3	9.3	85.1	79.2	5.9
G6	99	0.858	0.804	0.054	89.7	82.8	6.9	82.0	80.5	1.5
T3	94	0.967	0.908	0.059	97.9	91.5	6.4	92.4	89.7	2.7
W6	96	0.730	0.635	0.095	76.7	67.4	9.3	75.7	NA	NA
I1	63	0.867	0.719	0.148	97.1	85.3	11.8	79.3	72.4	6.9
I3	63	0.946	0.724	0.222	100	75.9	24.1	83.9	74.0	9.9

^a See Table 2 of the online Data Supplement for allergen codes.

^b Maximum sensitivity is the highest clinical sensitivity achievable by the assay for that allergen.

^c Also known as Q*, the highest value that sensitivity and specificity can jointly attain.

^d NA, not applicable (below the 0.35 kIU/L lower limit of the CAP working range).

for definitive clinical approaches such as challenge testing (6). Despite many potential discrepancies between the in vivo and in vitro procedures (26), laboratory systems for sIgE determination must demonstrate acceptably high concordance with objective clinical tests, as did the IML assay in the present study with skin testing as the objective clinical comparison method. Discrepancies between the IML and CAP assays were also resolved. Future studies on the performance of the IML assay could address allergens not investigated here, as well as different prospective study protocols ideally involving challenge testing to determine clinically relevant cutoffs and the quantitative relationship between clinical reactivity and sIgE based on IML data (30).

In conclusion, laboratory testing for sIgE in atopic or immediate-type allergies can be accomplished on a fully automated, random-access immunoanalyzer, such as the IML, at a diagnostic accuracy relative to clinical skin testing comparable to that of the widely used CAP technology for in vitro sIgE measurement.

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